

Please amend the specification as follows:

In the specification:

Please amend the title as follows:

-- ~~DNA ENCODING A NOVEL RG1 POLYPEPTIDE~~ METHODS FOR DETECTING
DISEASE-STATE ASSOCIATED WITH RG1 POLYPEPTIDE EXPRESSION --

Please amend the specification at page 1, lines 4-6 as follows:

-- This application is a continuation-in-part application of U.S. Application Serial No. 09/732,357, filed December 7, 2000, now U.S Patent No. 6,682,902, and which claims the benefit of U.S. Provisional Application No. 60/172,370, filed December 16, 1999, all of which is are incorporated herein in full by reference in their entirety.--

Please amend the paragraphs at page 8, lines 3-9 as follows:

-- FIGURE 9: Amino acid sequence of the variable chain regions of human monoclonal antibody B, including a mutated variable heavy chain region. V_L (SEQ ID NO: 26), V_H (SEQ ID NO: 27), $V_{H_2mB_3M}$, V_H (SEQ ID NO: 28).

FIGURE 10: Amino acid sequence of the variable chain regions of human monoclonal antibody C, including a mutated variable heavy chain region. V_L (SEQ ID NO: 29), V_H (SEQ ID NO: 30), $V_{H_3mC_2m}$, V_H (SEQ ID NO: 31). --

Please amend the paragraph on page 26, lines 11-22 as follows:

-- The following vectors, which are commercially available, are provided by way of example. Among vectors preferred for use in bacteria are pQE7O, pQE6O and pQE-9, available from Qiagen USA (Valencia, CA); pBS vectors, Phagescript® PHAGESCRIPT® vectors, Bluescript® BLUESCRIPT® vectors, pNH8A, pNHI6a,

pNHI8A, pNH46A, available from Stratagene (LaJolla, CA); and ptrc99a, pK223-3, pKK233-3, pDR54O, pRIT5 available from Pharmacia Biotech (Piscataway, N.J.). Most preferred is the pTrcHisB vector, available from Invitrogen. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, PXTI and pSG available from Stratagene; and PSVK3, pBPV, pMSG and pSVL available from Pharmacia Biotech. Most preferred is the pCneo vector available from Promega. These vectors are listed solely by way of illustration of the many commercially available and well known vectors that are available to those of skill in the art for use in accordance with this aspect of the present invention. It will be appreciated that any other plasmid or vector suitable for, for example, introduction, maintenance, propagation or expression of a polynucleotide or polypeptide of the invention in a host may be used in this aspect of the invention.

Please amend the paragraph at page 27, lines 34-37 through page 28, lines 1-17 as follows:

-- For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. The signals may be endogenous to the polypeptide or they may be heterologous signals. The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals but also additional heterologous functional regions. Thus for instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, special regions also may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. For example, when large quantities of RG1 are needed for the induction of antibodies, vectors which direct high level

expression of fusion proteins that are readily purified may be desirable. Such vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as Bluescript®-BLUESCRIPT® (Stratagene), in which the *rg1* coding sequence may be ligated into the vector in frame with sequence for the amino-terminal Met and the subsequent 7 residues of β-galactosidase so that a hybrid protein is produced; pIN vectors (Van Heede and Shuster, *J. Biol. Chem.* 264:5503-5509, 1989) and the like. PTrcHis vectors (Invitrogen, Carlsbad, CA) may be used to express foreign polypeptides as fusion proteins containing a polyhistidine (6xHis) tag for rapid purification. Proteins made in such systems are designed to include cleavage sites, such as an enterokinase cleavage site, so that the cloned polypeptide of interest can be released from the fusion peptide moiety at will.--

Please amend the specification on page 34, lines 12-19 as follows:

-- Furthermore, "human" antibodies can be produced using the methods described in U.S. Patent Nos. 5,877,397 and 5,569,825, which are incorporated herein in full by reference, or through use of the *Xenomouse*™-*XENOMOUSE*™, as described in Mendez et al. *Nature Genetics* 15:146-156, 1997. Such antibodies can also be generated using phage display technology (Rader et al., *Current Opinion in Biotechnology* 8:155-168, 1997; Aujame et al., *Human Antibodies* 8:155-168, 1997). The generation of human antibodies is very attractive, in that such antibodies are expected to minimize the immunogenic and allergic responses intrinsic to mouse or mouse-derived monoclonal antibodies. Generation of human antibodies which recognize epitopes of the RG1 polypeptide (SEQ ID NO: 2) are described in Example 4. --

Please amend the specification at page 39, lines 28-37 as follows:

-- Genetic immunization methods may be employed to generate prophylactic or therapeutic humoral and cellular immune responses directed against cancer cells expressing RG1. Using the RG1-encoding DNA molecules described herein, constructs

comprising DNA encoding an RG1 polypeptide/immunogen and appropriate regulatory sequences may be injected directly into muscle or skin of an individual, such that the cells of the muscle or skin take up the construct and express the encoded RG1 polypeptide/immunogen. The RG1 polypeptide/immunogen may be expressed as a cell surface polypeptide or be secreted. Expression of the RG1 polypeptide/immunogen results in the generation of prophylactic or therapeutic humoral and cellular immunity against prostate cancer. Various prophylactic and therapeutic genetic immunization techniques known in the art may be used (for a review, see information and references published at internet address www.genweb.com). --

Please amend the specification on page 47, lines 3-10 as follows:

-- *Rg1* was identified as a gene expressed in the prostate by mining Incyte's *LifeSeq®* *LIFESEQ®* database. The nucleotide sequence was identified by an annotation search of the database, using the "Protein Function" tool provided by Incyte for the purpose of searching the database. The nucleotide sequence was found in the category of cell adhesion molecules in the annotated database and was described as a homologue of f-spondin. Electronic Northern analysis of the distribution of *rg1* polynucleotide sequences in the set of libraries in the database revealed that *rg1* was expressed at high levels in the prostate libraries and at lower levels in a number of other tissue libraries, including those from normal and tumor tissues. --

Please amend the specification on page 56, lines 23-33 as follows:

-- Mutagenesis: Site-directed mutagenesis of the wild-type cDNA encoding variable regions of anti-RG1 antibody B and C was carried out to generate allotypes that are more frequently expressed in humans. The *QuickChange®* Multisite-directed Mutagenesis mutagenesis was performed using a kit sold by Stratagene (QUICKCHANGE®) method

~~(Stratagene)~~ was used to conduct the mutagenesis, with TOPO/BVH and TOPO/CVH (Medarex) as templates. Primers (GGGGAGGCTTGGTACACCTGGGGGTCCCTGAG; SEQ ID NO: 14) and (GAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCAAG; SEQ ID NO: 15) were used to introduce the point mutations H13Q, M90T and M92V into B cDNA (BVH_3m); and H13Q, M90T into C cDNA (CVH_2m). Mutations were confirmed by DNA sequence analysis and resulted in the mutant heavy chain variable regions with sequences of SEQ ID NO: 22 and SEQ ID NO: 25, respectively. The predicted amino acid sequences for these two heavy chain variable regions are given by SEQ ID NOS: 28 and 31, respectively. --